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A. F. H. BAKER

AN ATTEMPTED SYNTHESIS

OF

THE CYANOGENETIC GLUCOSIDE

LOTAUSTRALIN.

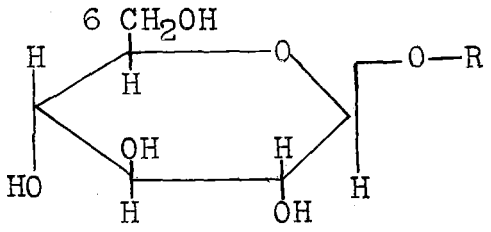
C O N T E N T S.

THEORETICAL

INTRODUCTION TO GLYCOSIDES.

The term"glycoside" is a general term applied to compounds containing a group combined with a sugar residue through an ether linkage involving the reducing hydroxyl. The non-sugar portion is called the aglycone.

The sugar portion may consist of residues of any of a number of sugars but glucose is most often present. In this case the glycoside is called a "glucoside" and possesses the following general formula:-



A β-D-glucopyranoside.

In general glycosides are colourless crystalline solids with a bitter taste. They are stable to dilute alkali but are hydrolysed by dilute acid or enzymes, the latter often being found in the same plant (if it is a plant glycoside). The rate and ease of hydrolysis varies widely. In optical activity they are often laevorotatory and their optical rotation remains constant in non-ionising solvents i.e. they do not mutarotate. Since they do not reduce Fehling's Solution or combine with phenylhydrazine or similar reagents, it is clear that the reducing hydroxyl group on carbon one is the one involved in the glycosidic link.

OCCURRENCE AND FUNCTION.

Glycosides are widely distributed in nature, occurring in plant and animal tissues.

Amongst the animal glycosides are the important nucleoproteins. These contain D-ribosides of purine bases and 2-deoxy-D-ribosides of pyrimidine bases, both of which are essential constituents of the cell nuclei of animals and plants.

In plants, glycosides may occur in almost any part of the organism but they are found especially in the roots, bark and fruit.

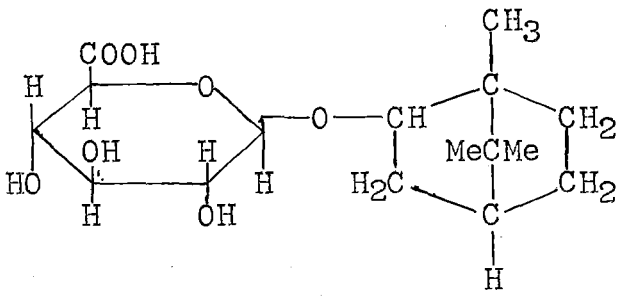
Where bitter glycosides are found in the bark or seed case, they are thought to be protective in function and that the liberation of hydrogen cyanide by hydrolysis of cyanogenetic glucosides in the bark may have an antiseptic action and so assist in the healing of wounds.

Glycosides may act as reserve materials in plants, being brought into the circulation by hydrolysis by a particular enzyme when they are needed. Many colouring matters exist in the plant as glycosides.

The amount of glycoside present varies with the species of plant and the time of year.

DETOXIFICATION.

Detoxification is the process by which toxic substances are rendered harmless in the animal body. This is a very common process and frequently occurs by a type of glycoside formation. Borneol, for instance, combines with glucuronic acid through carbon atom one to give a glucuronide.



Borneol Glucuronide.

EXTRACTION.

Extraction is usually effected with water or alcohol in some apparatus like a Soxhlet extractor.

Superheated steam may be used to destroy enzymes which are often present in a different part of the same plant.

CLASSIFICATION.

Glycosides are usually classified according to their aglycones, e.g. the red and blue pigments of flowers form a group, the anthocyanins, all containing anthocyanidins combined with sugars and the cyanogenetic glycosides all possess a cyanide group in the aglycone.

STRUCTURE.

The structure of glycosides may be discussed under the following headings:-

(a) The sugar or sugars present.

(b) The aglycone.

(c) The type of linkage i.e. whether α or β .

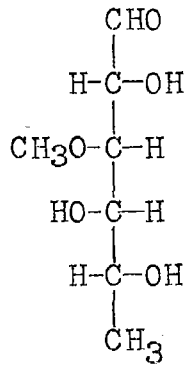
and (d) The ultimate proof of structure by synthesis.

(a) The Sugar or Sugars Present.

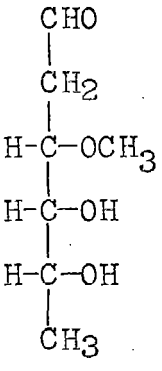
The sugar most often found is D-glucose but many others

occur e.g. rhamnose (ω-deoxy-L-Mannose) is frequently present and xylose is common. In the nucleosides D-ribose and 2-deoxy-D-ribose occur[1] and the cardiac glycosides contain a number of unusual sugars[2] e.g.

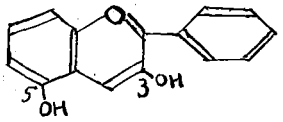
Digitalose
3-methyl-6-deoxy-
D-galactose



Cymarose
3-methyl-2,6-deoxy-
D-allose
or
3-methyl-2,6-deoxy-
-D-altrose



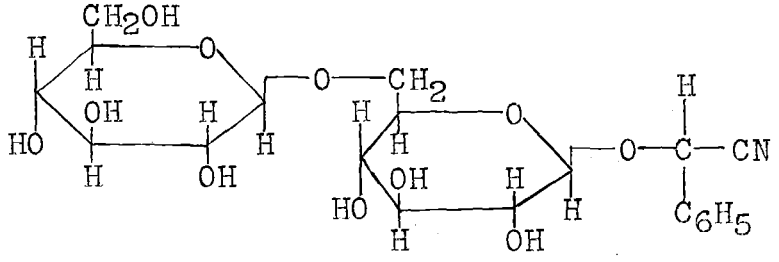
When two or more monosaccharide residues are present, as indicated by the isolation from the hydrolysis mixture of two or more molecules of monosaccharide per molecule of glycoside, they may be attached to separate positions on the aglycone e.g. positions 3 and 5 in the anthocyanin glycosides[3].



The Anthocyanidin Nucleus.

Alternatively the monosaccharide molecules may be combined as a disaccharide or trisaccharide.

e.g. Amygdalin[4].

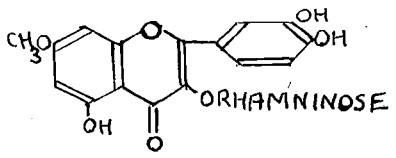


β-Gentiobiose Mandelonitrile.

The enzymic hydrolysis of glycosides is a reversible reaction and its rate is retarded by the addition of only one sugar, namely that present in the glycoside.

A number of equal portions of a glycoside solution is taken and to each is added an equal volume of an enzyme solution and a definite quantity of a number of sugars. After a short time, say two hours, the enzyme is destroyed and the extent of hydrolysis determined.

In one experiment the glycoside xanthorhamnin



Xanthorhamnin.

was hydrolysed to the extent of 55 - 60% either alone or with glucose, fructose, galactose, rhamnose, sucrose, lactose, maltose or raffinose. However, in the presence of rhamninose (a trisaccharide containing one galactose and two rhamnose residues per molecule) which is the sugar formed during hydrolysis, only 39% of the glycoside was decomposed. This is the basis of Ter Meulen's Method for finding the sugar present in a glycoside[5].

The sugars may also be characterised from the hydrolysis mixture as any of their normal derivatives e.g. semicarbazones phenylhydrazones, osazones, or osotriazoles.

The formation of phenyl-osotriazoles is a recently developed method of great promise in the characterising of carbohydrates. The phenyl osazone is converted to the phenylosotriazole by boiling with aqueous copper sulphate.

Hann and Hudson who discovered this reaction[6] state that "the phenylosotriazoles have very low solubilities in water, crystallise spontaneously, are stable, and have sharp melting points, specific rotations and crystalline appearance". They recommend the method for small samples as the yields are high.

THE POSITION OF THE SUGAR RESIDUE.

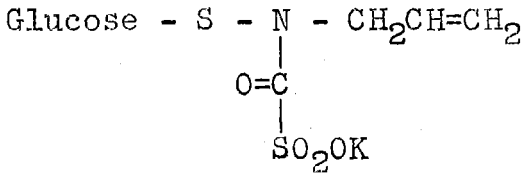
The position of the sugar residue in the aglycone may be determined by methylating the glycoside and then hydrolysing the methylated product with dilute acid or an appropriate enzyme. The position of the free hydroxyl is then determined. Diazomethane can be used to methylate the phenolic hydroxyl groups in glycosides of the flavone class.

The monosaccharide residues are present in the pyranose or six membered type of ring with the exception of D-ribose and 2-deoxy-D-ribose in nucleosides, the unusual sugars in the cardiac glycosides and fructose in oligosaccharides, all of which exist in the furanose or five membered ring form. The evidence for the type of ring is based on oxidation of methylated sugars and the properties of the methylated aldonic acid lactones[7]. Evidence from X-ray analysis[8] in the solid state confirmed the accepted configurations of α and β glucose and showed that the carbon atoms in the ring are coplanar and do not form Sachse strainless rings. The oxygen atom is slightly displaced out of the ring. It is thought that the structure is the same in solution also.

(b) THE AGLYCONE.

The non-sugar portion of glycosides varies from the simplest alkyl group to the most complex steroid genins of the cardiac glycosides.

The free aglycone is almost always an hydroxy compound. In the nucleosides, however, the sugar is joined to the nitrogen atom in position nine in the purine nucleus and to the nitrogen atom in position three in the pyrimidine ring[1]. In the mustard oil glycosides the sugar is joined directly to a sulphur atom.

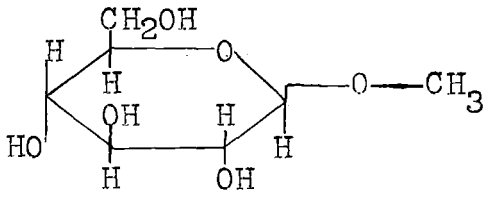


Sinigrin (Potassium myronate).

(c) THE STEREOCHEMISTRY OF THE GLYCOSIDIC LINKAGE.

With only one or two dubious exceptions all the naturally occurring glycosides have the beta configuration of the linkage between the sugar and aglycone.

In the common glucosides (β-glucosides) the aglucone occupies a position on the opposite side of the ring from the hydroxyl group on carbon two.



β-methyl-d-glucopyranoside.

Evidence for the configuration of the hydroxyl on carbon one of glucose itself was early obtained from the measurement of the electrical conductivity of solutions of the sugar in the presence of boric acid[9].

It had been observed that the addition of boric acid to a solution of a glycol increased the conductance more when the adjacent hydroxyls possessed the cis configuration than when they were trans with respect to each other.

Applying this rule to his results for glucose Boeseken concluded that in the conventional α form the glucosidic hydroxyl lies on the same side of the ring as the hydroxyl on carbon two.

The rates of the gradual fall in conductivity of the α-D-glucose-boric acid complex solution and the rising conductivity of the β-D-glucose-boric acid complex solution coincided with the rate of mutarotation.

The relationship between α- and β-glucose and α- and β-glucosides was shown by Armstrong[10]. He showed that in the hydrolysis of α-methyl-D-glucoside with maltase, the enzyme from yeast, the addition of a drop of ammonia after the reaction had proceeded for a short time, caused a marked fall in optical rotation thus proving that it was α-D-glucose which was formed. Similarly using β-methyl-D-glucoside and emulsin from bitter almonds, the specific rotation increased on the addition of ammonia. This shows that α-D-glucose results from the enzyme hydrolysis of α-methyl-D-glucoside. The ammonium hydroxide caused the rapid attainment of the

equilibrium between the α and β forms i.e. mutarotation.

The above proof was possible only because hydrolysis proceeds faster than mutarotation before the addition of the ammonia.

MUTAROTATION.

A solution of either an alpha or beta sugar with a free reducing hydroxyl in an ionising solvent changes in optical rotation, i.e. mutarotation, until it attains a constant value.

The alpha or beta glycosides, however, can be interconverted only with great difficulty, if at all.

The conversion of β-methyl-tetraacetyl-glucoside into the α isomer was accomplished by Pacsu[11] by heating with $TiCl_4$ in a chloroform solution. The conversion was almost quantitative and there was little decomposition.

HUDSON'S ISOROTATION RULES.[12]

Hudson assumed that the principle of optical superposition holds for sugars. It states that in a molecule with a number of centres of asymmetry, the contribution to the total optical rotation made by the remainder of the molecule is independent of the configuration about the end carbon atom.

The molecular rotation of the end carbon atom may be designated A and that of the rest of the molecule B. For one isomer then, the molecular rotation is equal to A + B, while for the other, it is -A + B.

The molecular rotation of the alpha form of a sugar minus that of the beta form is equal to 2A and should be constant for a series of free sugars or sugars with the same substituent on carbon one.

He showed that this was approximately true. Pigman[12] has shown (1941), however, that the 2B value for glucose is greatly increased if an aromatic ring constitutes the aglucone.

	2A (the difference)
α-and β-D-glucose	+ 16,000
α-and β-D-galactose	+ 15,700
α-and β-D-lactose	+ 17,400
α-and β-L-arabinose	- 16,200
α-and β-methyl-D-glucoside	36,700
α-and β-methyl-d-galactoside	37,900

From this work he deduced a method for naming the alpha and beta isomers superior to the old arbitrary one of calling the more detrorotatory isomer alpha and the other beta.

He formulated the rule that for sugars and glucosides genetically related to D-glucose, the subtraction of the optical rotation of the β-isomer from that of the α-isomer should give a positive difference, but for those sugars and glycosides related to L-glucose this difference should be negative.

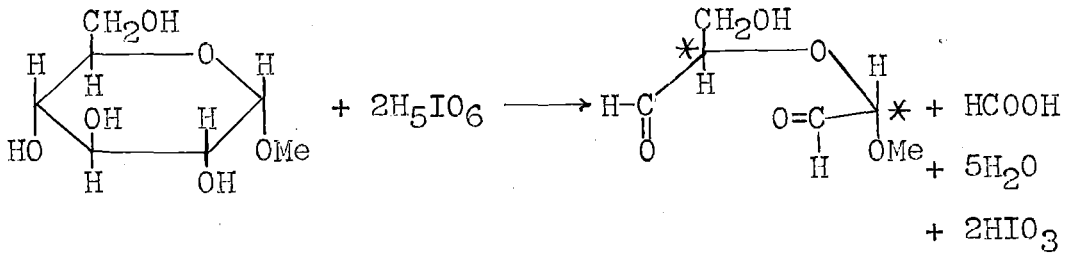
(a) BY BROMINE OXIDATION.

Isbell and Pigman[13] distinguish alpha and beta sugars by comparing their rates of oxidation by bromine. They have shown that the beta isomer is more rapidly oxidised

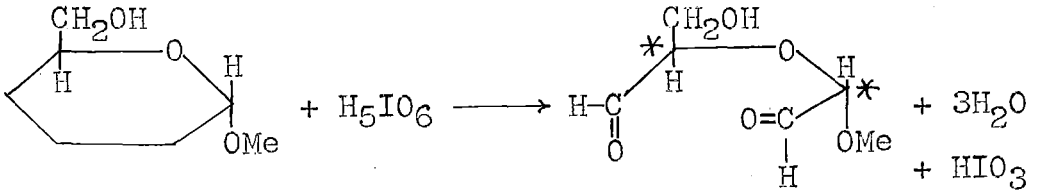
than the alpha form in the pentoses, hexoses, and heptoses which have been examined.

(b) BY PERIODIC ACID OXIDATION.[14]

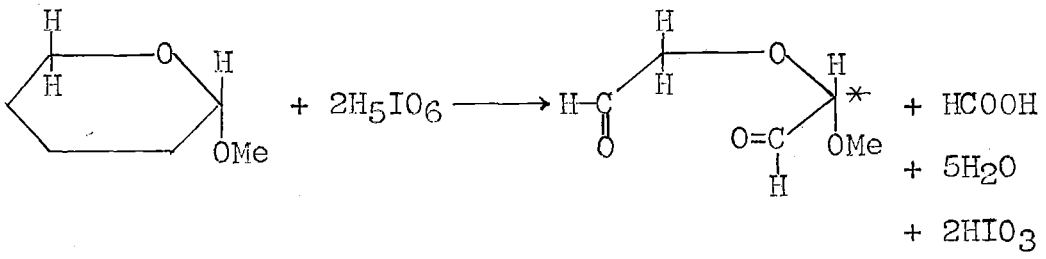
Jackson and Hudson showed that when a glycoside is treated with a solution of periodic acid (H_5IO_6) in water, oxidative rupture of the ring occurs giving an optically active diglycollic aldehyde.



∝-methyl-D-glucopyranoside. D'- methoxy-D-hydroxymethyl -diglycollic aldehyde.



∝-methyl-D-pentofuranoside. D'-methoxy-D-hydroxymethyl -diglycollic aldehyde.



Any methyl-D-pentopyranoside. D'-methoxy-diglycollic aldehyde.

Two adjacent hydroxyls are oxidised to aldehyde groups with fission of the carbon to carbon bond but if hydroxyls are present on three or more adjacent carbon atoms those at

the ends are oxidised to aldehyde groups as usual while those in between are eliminated as formic acid.

One molecule of periodic acid is used in splitting the glycol carbon to carbon bond and one additional molecule is required for every (CHOH) group above two in number if they are adjacent. The amount of periodic acid used therefore, gives direct evidence on the size of the ring. Pyranose rings all use two molecules of periodic acid per molecule of glycoside, while furanosides require only one. A glycoside with the septanose ring would be expected to consume three molecules of periodic acid but it appears that this has not been confirmed experimentally yet.

The diglycollic aldehydes produced in the oxidation are optically active due to the asymmetry of carbon one of the sugar and of carbon five also, in aldohexopyranosides. The configuration of the aldehydes derived from all the aldohexopyranosides and aldopentofuranosides should be the same if the sugars are related to D-glyceraldehyde and the glycosidic group is consistently either alpha or beta. The aldehyde derived from the aldopentopyranosides or aldohexoseptanosides (containing the septanose or seven membered ring) would contain only one centre of asymmetry, that due to carbon one. This means that their α-L- and α-D-glycosides give the same aldehyde.

Jackson and Hudson[14] (1937) verified the above statements by treating a number of methyl glycosides with periodic acid and isolating the pure aldehydes.

	Dialdehyde $[\alpha]_D^{20°}$	
α-methyl-D-xylopyranoside	+ 125.2	
β-methyl-D-xylopyranoside	- 124.3	
α-methyl-D-arabinofuranoside	+ 117.3	(slightly impure)
α-methyl-D-glucopyranoside	+ 121.1	
α-methyl-D-galactopyranoside	+ 120.7	
β-methyl-D-galactopyranoside	- 148.1	
β-methyl-D-glucopyranoside	- 150.6	

This method provides an elegant means of proving the ring structure and the configuration of groups about carbon one in a simple and direct way.

(c) THE ACTION OF ENZYMES.

Some enzymes are specific in their hydrolysing action on the two types of linkage. For example emulsin from bitter almonds hydrolyses β-methyl-D-glucopyranoside but does not affect α-methyl-D-glucopyranoside.

The rule that only β-glucosides are hydrolysed by emulsin is not without exceptions and in general enzymic hydrolysis of a glycoside does not provide certain information on the configuration of the glycosidic link. In a number of glycosides emulsin is without effect on both alpha and beta isomers.

The ease of enzymic hydrolysis of glycosides varies for one particular sugar with the nature of the aglycone. Fischer[15] found that derivatives of α-hydroxy-butyric acid are very resistant to hydrolysis and in line with this Finnemore and Cooper[16] noted that Lotaustralin is only slowly acted on by almonds i.e. emulsin.

(d) THE SYNTHESIS OF GLYCOSIDES.

Synthesis is regarded as the ultimate proof in establishing the structure of a glycoside. The more important methods of synthesis are briefly reviewed in this section.

In 1893 Fischer prepared a number of hemiacetals among which was ∝-methyl-D-glucopyranoside[17]. He treated the free sugar with anhydrous methanol saturated with hydrogen chloride. In 1914 he prepared "γ-methyl glucoside" in impure form by condensing glucose with methanol in the presence of hydrogen chloride at low temperature[18]. This "γ-methyl glucoside" was shown to be methyl-D-glucofuranoside by Haworth[19] in 1926, and pure crystalline ∝-methyl-D-glucofuranoside was prepared[20] by Haworth, Porter and Waine in 1932. From the above work it is seen that if glucose is treated with dry methanol in the presence of 1% dry hydrogen chloride, the product is mainly methyl-D-glucofuranosides when the reaction is carried out at room temperature. If, however, the mixture is heated under reflux the pyranoside modification is the main product.

This reaction is a general one and can be applied to other sugars and to a variety of alcohols viz. ethyl, propyl, benzyl and similar alcohols.

THE DIMETHYL SULPHATE METHOD.

Maquenne (1905) showed that glycosides can be prepared by the careful methylation of the sugar with one equivalent of dimethyl sulphate i.e. $(CH_3)_2SO_4$ and sodium hydroxide in water[21].

THE PURDIE-IRVINE METHOD.

Purdie and Irvine developed a method by which methylated sugars can be prepared by the action of silver oxide and methyl iodide on the free sugar[22] but the hydroxyl group on carbon one tends to be oxidized by the silver oxide.

The above methods due to Maquenne and Purdie and Irvine are not very important for preparing methyl glycosides but they are the standard reactions for producing completely methylated sugars.

SUGAR ANHYDRIDES IN GLYCOSIDE SYNTHESIS.

1:2-anhydro-3:4:6-triacetyl-glucosan on treatment with aliphatic alcohols or with phenols, gives glycosides by opening of the ethylene oxide ring. β-glycosides result with aliphatic alcohols but phenol forms α-phenyl-D-glucoside[23].

THE KOENIGS-KNORR REACTION.

This is the most important method of synthesising complex glycosides. Although it was first used by Michael[25] in the synthesis of Methyl Arbutin in 1881, it is generally known by the above name as these investigators established it as a standard method. The reaction makes use of the discovery that in a fully acetylated sugar the acetyl group on carbon one is more reactive than the others and can be replaced by an atom of a halogen (usually bromine) on treatment with a solution of the dry halogen halide in glacial acetic acid.

The halogen is very reactive in the case of iodine and there is a marked decrease in its reactivity in passing from

iodine up to fluorine[26].

The acetohalogen sugar will condense with hydroxy compounds with the elimination of hydrogen halide and the formation of a tetraacetyl-glycoside. Where the sugar is to be directly linked to a sulphur atom as in the mustard oil glycosides or nitrogen, as in the purine or pyrimidine glycosides, a silver or potassium salt of the aglycone may be reacted with the acetohalogen sugar but in most reactions silver oxide or carbonate is used to remove the hydrogen halide and the addition of a drying agent has been found to improve the yield.

Since carbon atom one is asymmetric there is usually a Walden inversion of configuration on it when the bromine is replaced. With glucose and most other sugars it is fortunate that the more stable of the two isomeric acetyl-glycosyl-halides is that with the alpha configuration[27] so that when this isomer reacts in the Koenigs-Knorr reaction Walden inversion occurs with the formation of the β-acetyl-glycoside only. This is usually the more important compound. It has been observed, however, that α-acetochloromannose[28] and α-acetobromorhamnose[29] give mixtures of alpha and beta acetylated glycosides as well as orthoesters.

In 1916 Fischer discovered that by substituting quinoline for silver oxide as the means of removing the hydrogen halide, the Koenigs-Knorr reaction could produce a mixture of alpha and beta isomers[30]. It has also been observed[21] that up to 90% alpha can be prepared by using 2-trichloracetyl-3:4:6-triacetyl-β-D-glucosyl chloride in the ordinary reaction.

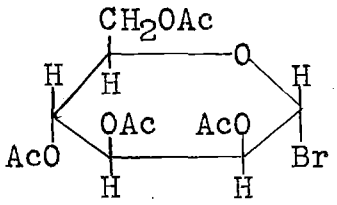
The following solvents are commonly employed, dioxan, benzene, ether, acetone or an excess of the aglycone itself if it is a liquid. The hydrogen halide has been removed with silver oxide or carbonate, potassium hydroxide, pyridine or quinoline. The addition of "Drierite" (anhydrous calcium sulphate) or anhydrous magnesium sulphate improves the yield probably by suppressing the side reaction[32] between the halogen and water, in which the halogen is replaced by a hydroxyl group.

THE ZEMPLEN MODIFICATION OF THE KOENIGS-KNORR REACTION.

Zemplen found in 1929[33] that an acetobromo-sugar on treatment with phenol in the presence of aluminium filings and mercuric acetate in benzene produced the acetylated α-phenyl glycoside. The following year he showed that the aluminium was unnecessary[34]. In this reaction α-glycosides are formed when the concentration of the aglycone is low and β-glycosides when it is high compared with that of the acetobromosugar. A series of experiments with varying quantities of mercuric acetate indicated that for maximum yields of glycoside the concentration of the mercury salt should be less than equivalent to the hydrogen bromide formed[34]. In 1947 Zemplen[35] used the completely acetylated sugar and mercuric bromide instead of the acetobromosugar and mercuric acetate.

ORTHOESTER FORMATION.

If the halogen atom on carbon atom one is trans with respect to the acetyl group on the second carbon atom as in ∝-tetraacetyl-D-mannopyranosyl bromide,



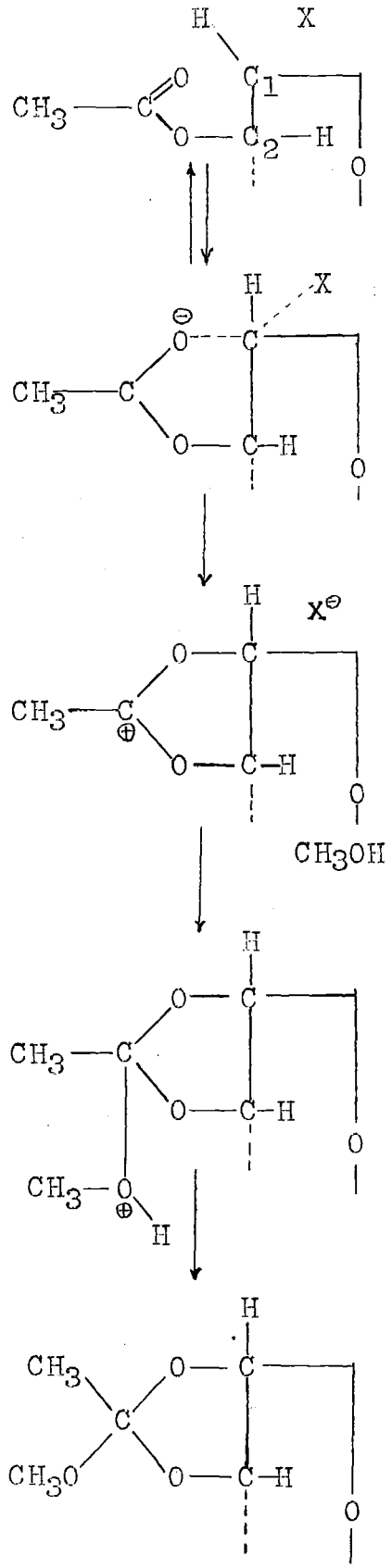
∝-tetraacetyl-D-mannopyranosyl bromide.

then an orthoester forms as well as some β-tetraacetyl-glycoside on treatment with an alcohol and silver oxide. The reaction is a competitive one and the proportion of orthoester and alpha- and beta-glycoside derivatives produced is determined by the experimental conditions employed[36].

Frush and Isbell showed that the trans configuration of acetyl and halogen permitted an "opposite face attack" on carbon one by the carbonyl oxygen atom of the acetyl group on carbon two. The halogen is simultaneously eliminated as an anion. The intermediate cation so formed reacts with a molecule of alcohol to give the orthoester. It was early observed that the acetyl group involved in orthoester formation is stable to dilute alkali but is hydrolysed by dilute acid.

Orthoesters have been reviewed recently by Pacsu who quotes the following mechanism in "Advances in Carbohydrate Chemistry" Vol. I.

POSTULATED MECHANISM FOR ORTHOESTER FORMATION.



The acetyl group and halogen are trans.

A methyl orthoester.

GLYCOSIDES FROM SUGAR MERCAPTALS.

In 1925 Pacsu[37] showed that when sugar mercaptals are boiled with an excess of mercuric chloride in absolute alcoholic solution α-alkyl-pyranosides are produced in high yield.

$$C_6H_{12}O_5(SCH_2Ph)_2 + 2HgCl_2 + C_2H_5OH \rightarrow 2HCl + 2PhCH_2S\text{-}Hg\text{-}Cl + C_6H_{11}O_5\text{-}OC_2H_5$$

Hexose dibenzyl-mercaptal — α-Ethyl-pyranoside.

In 1937 Green and Pacsu[38] modified the previous experiment by adding excess yellow mercuric oxide and allowed the reaction to proceed at room temperature. Mainly β-furanoside was formed but about 2% of the alpha isomer was isolated.

If the mercaptal is treated with mercuric chloride and yellow mercuric oxide in aqueous solution at 0°C. the thiofuranoside[39] is formed.

THE SYNTHESIS OF PHENOLIC GLYCOSIDES.[40]

Helferich has prepared phenolic glycosides by heating either alpha or beta fully acetylated sugars with the phenol. In the presence of anhydrous zinc chloride the formation of the alpha isomer is favoured but para-toluene-sulphonic acid favours the beta derivative.

THE ENZYMIC SYNTHESIS OF GLYCOSIDES.

The enzymic hydrolysis of a glycoside is a reversible reaction.

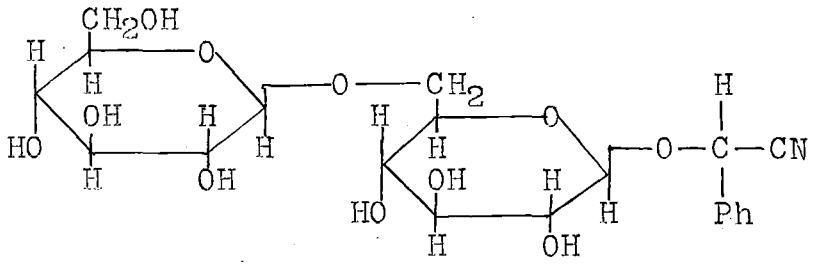
$$\text{Glycoside} + \text{water} \rightleftharpoons \text{sugar} + \text{aglycone}.$$

In dilute solutions the equilibrium lies almost completely to the right but in higher concentrations of the aglycone e.g. an alcohol, the same glycoside can be synthesised. This has been called the Biochemical Synthesis of glycosides. By this means α-methyl glucoside was synthesised through the agency of yeast glucosidase[41]. The same experimenters also produced α-allyl- and α-propyl-glucosides by the same means.

THE CYANOGENETIC GLYCOSIDES.

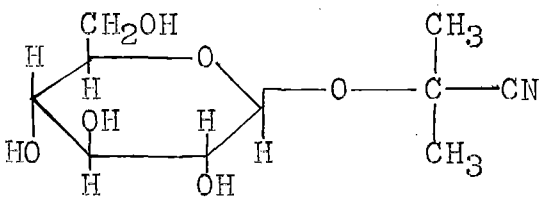
Armstrong states that when hydrogen cyanide can be isolated from plant tissues its origin can invariably be traced to a glycoside. These glycosides containing a nitrile group in the aglycone are called "Cyanogenetic" or "Cyanophoric" glycosides. One of the best known members of this group is Amygdalin found in bitter almonds and the kernels of some stone fruit. It was discovered in 1830 but was not synthesised until almost a hundred years later.

It is mandolonitrile-β-gentiobioside.



Amygdalin.

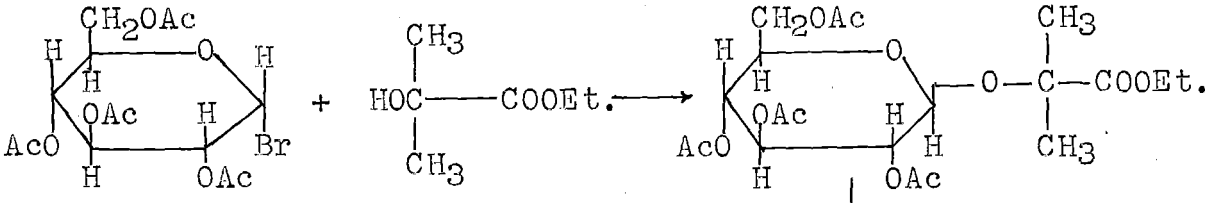
Another fairly well known glycoside in this class is Linamarin, present in flax and in the seeds of the rubber tree. It is the glucoside of acetone cyanhydrin.



Linamarin.

The following chart shows the method used by Fischer and Anger in their synthesis of Linamarin[42] in 1918. A similar synthesis was used by Campbell and Haworth[43] in their synthesis of Amygdalin in 1924.

THE SYNTHESIS OF LINAMARIN.

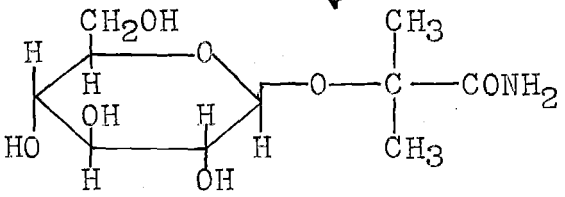


∝-acetobromoglucose

Ethyl-∝-hydroxyisobutyrate

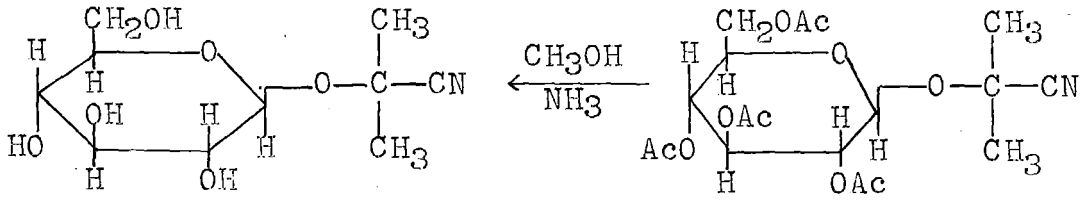
CH_3OH-NH_3

Methanolic ammonia



This compound was reacetylated by means of Ac_2O and pyridine.

$POCl_3$



Linamarin.

PRESENT INVESTIGATION.

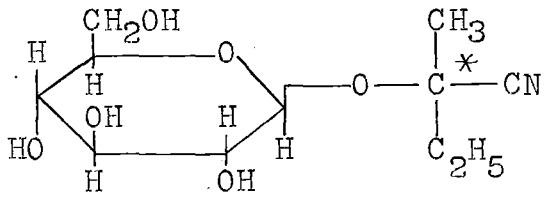
The object of the present investigation was to synthesise Lotaustralin, a cyanogenetic glucoside found in white clover, (Trifolium repens) and in Lotus australis. The structure of Lotaustralin has not been definitely established yet but the following evidence due to Finnemore, Cooper and Cobcroft is pertinent[16].

1. On hydrolysis with barium hydroxide it gave the barium salt of α-hydroxy-α-methyl-butyric acid.

2. With 10% hydrochloric acid methyl ethyl ketone was produced and hydrogen cyanide was evolved.

3. From the products of enzymic hydrolysis glucose was characterised as the osazone. A little acetone was also identified in the hydrolysis products suggesting that a small amount of Linamarin occurs naturally with the Lotaustralin isolated from Lotus australis.

The above data led to the postulation of the following formula for Lotaustralin by Finnemore, Cooper and Cobcroft[16]:



Lotaustralin.

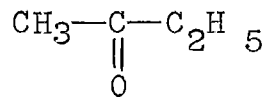
A sample from Lotus australis[16] was analysed by the above workers.

Found	C	49.56%	H 7.36%	
Theoretical	C	50.58%	H 7.28%	for $C_{11}H_{19}O_6N$.

m.p. 128° to 136°C and $[\alpha]_D^{20^\circ}$ - 26.37 (C= 1 in water)

In the present investigation an attempt has been made to synthesis Lotaustralin by a method closely parallelling that used by Fischer and Anger in their synthesis of Linamarin[42] and by Campbell and Haworth in the synthesis of Amygdalin[43] but the synthesis was not carried to completion.

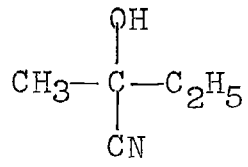
CHART OF PROPOSED SYNTHESIS.



Methyl ethyl ketone.

I.

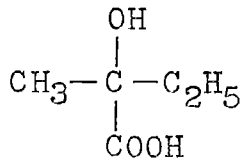
HCN and about 5 c.c. of saturated NaCN in water at 0° C.



dl-methyl-ethyl-ketone-cyanhydrin.

II.

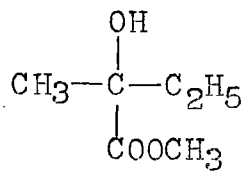
Concentrated hydrochloric acid at room temperature for some days.



dl-methyl-ethyl-glycollic acid.

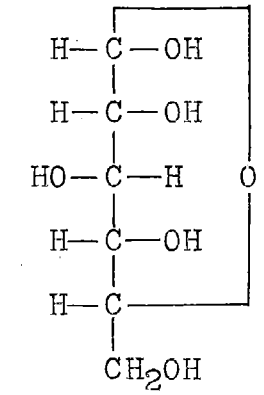
III.

Anhydrous $CuSO_4$ and CH_3OH refluxed.



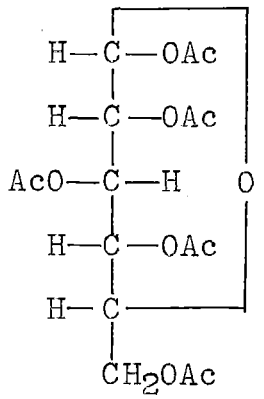
(A.) dl-methyl-α-hydroxy-α-methyl butyrate.

CHART OF PROPOSED SYNTHESIS (Contd.)



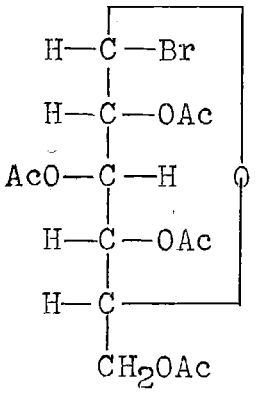
∝-D-glucose.

IV. Ac_2O and three drops of concentrated H_2SO_4 catalyst or 1:1 glacial Acetic acid : Ac_2O and 1.5 c.c. $HClO_4$ at room temperature.



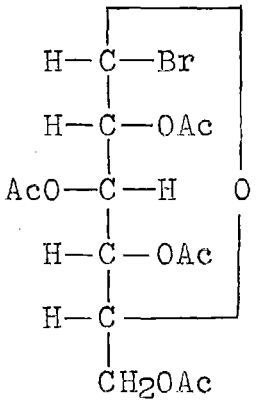
∝-1:2:3:4:6-pentaacetyl-D-glucose.

V. Glacial HAc saturated with HBr at 0° C.

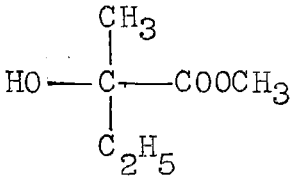


(B) ∝-2:3:4:6-tetraacetyl-glucosyl bromide.
(acetobromoglucose)

CHART OF PROPOSED SYNTHESIS (Contd.)



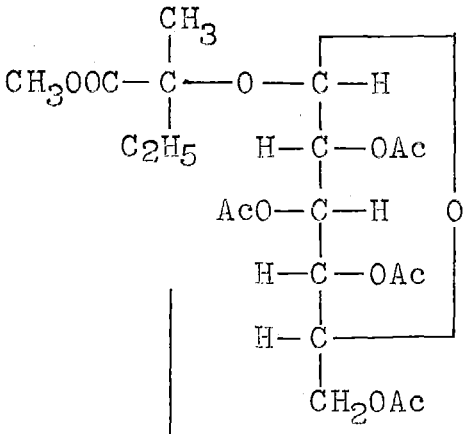
(B)



(A)

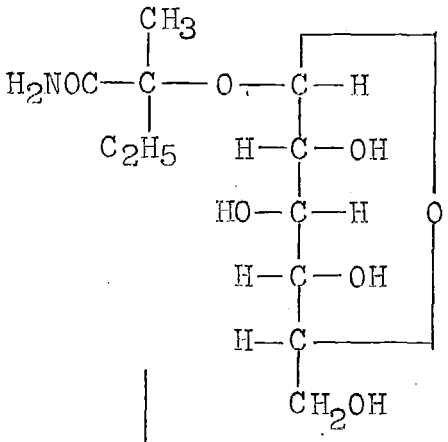
VI. Ag_2O and anhydrous $MgSO_4$.

Walden Inversion occurs in this reaction.



Condensation product.

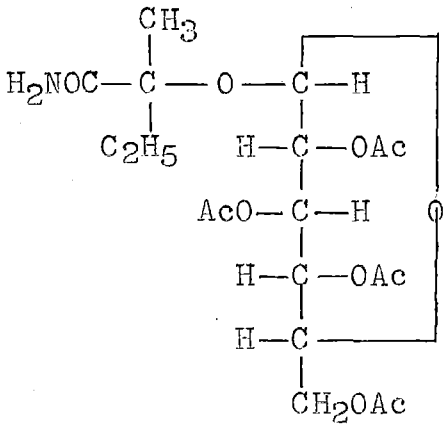
VII. Anhydrous CH_3OH saturated with NH_3 at 0° C.



The deacetylated amide.

VIII. Ac_2O and Pyridine at room temperature.

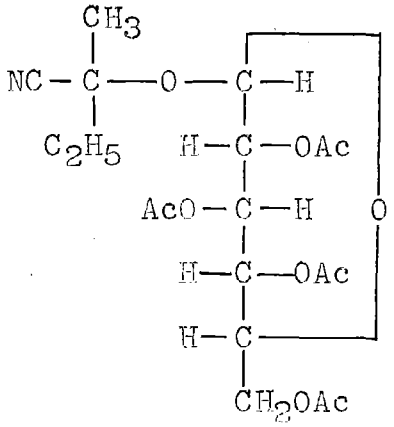
CHART OF PROPOSED SYNTHESIS (Contd.)



The acetylated amide.
d and l forms separated here.

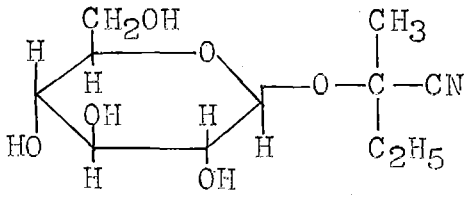
ONE ISOMER

IX. P_2O_5 in xylene or $POCl_3$ in pyridine.



Acetylated Lotaustralin.

X. Anhydrous CH_3OH saturated with NH_3 at 0^oC.



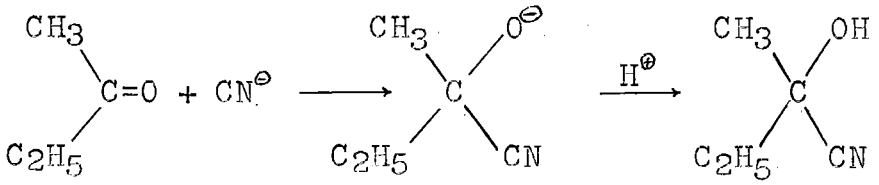
Lotaustralin.

A DISCUSSION OF THE REACTIONS IN THE PROPOSED SYNTHESIS.

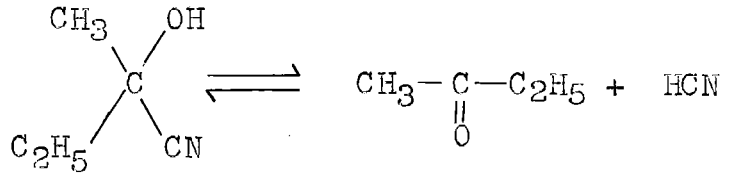
REACTION I. CYANHYDRIN FORMATION.

Two methods were used for the preparation of methyl ethyl ketone cyanhydrin. They differ in experimental details only, both relying on the direct addition of hydrogen cyanide to the ketone at $0^{o}C$. in the presence of a trace of sodium cyanide i.e. cyanide ions. Equilibrium is attained rapidly under these conditions and the low temperature favours a high conversion to cyanhydrin.

MECHANISM[44].



Lapworth and Manske[44,45] determined the dissociation constants of methyl ethyl ketone cyanhydrin and others in 96% alcohol at 20^{o} C. For the reaction



$K = 2.65 \times 10^{-2}$ and $\Delta G = -2.1$ kilo. cals.

This shows that the cyanhydrin is thermodynamically unstable at ordinary temperatures so that its purification should be carried out at as low a temperature as possible.

REACTION II.

THE PREPARATION OF α-HYDROXY-α-METHYL-BUTYRIC ACID.

The hydrolysis of nitriles to carboxylic acids is a well known reaction which may be carried out with either acid or alkali. The amide is the intermediate.

$$R\text{-}C\equiv N \xrightarrow{H_2O} R-\underset{\underset{O}{\|}}{C}-NH_2 \xrightarrow{H_2O} R-\underset{\underset{O}{\|}}{C}-OH + NH_3$$

With strong sodium hydroxide it was found in the present investigation that the methyl ethyl ketone cyanhydrin on heating gave an evil smelling solution with the odour of an isonitrile and on acidification no acid could be extracted with ether. Hydrolysis with strong hydrochloric acid[46] gave excellent yields of acid.

REACTION III.

THE PREPARATION OF METHYL-α-HYDROXY-α-METHYL-BUTYRATE.

1. An attempt was made here to convert the cyanhydrin to the ester directly by low temperature alcoholysis (i.e. at reflux temperature) with anhydrous alcoholic hydrogen chloride.

$$R—CN + R'OH + HCl \longrightarrow R—\underset{\underset{O}{\|}}{C}—OR'$$

Spiegel[47] using ten moles. of alcohol and one mole. of sulphuric acid per mole. of nitrile got high yields by heating in a sealed tube at 130° to 140°c. The above low temperature method failed. On neutralisation of the excess hydrogen chloride with silver carbonate and heating at 40°c at low pressure to remove the excess alcohol a white amorphous precipitate formed which charred but did not melt at 200° C.

It may have been lactide of the hydroxy acid. No ester distilled over.

2. Diazo-methane[48] prepared from N-methyl-nitrosourea was used to prepare the methyl ester. This was found to be an elegant method for small quantities, but was inconvenient for larger amounts.

$$RCOOH + CH_2N_2 \longrightarrow RCOOCH_3 + N_2$$

A great advantage lies in the almost complete absence of byproducts. Pure diazo-methane is explosive and is poisonous[49] even in very low concentrations. It was thought early in the present investigation that the ester gave rise to the lactide by a process similar to the formation of diketopiperazines from amino acid esters. Later it was found that the ester was fairly stable in the absence of water[50].

3. Anhydrous copper sulphate[51] acts both as a dehydrating agent and as a catalyst in the esterification, particularly of hydroxy acids.

REACTION IV. THE ACETYLATION OF GLUCOSE.

Anhydrous ∝-D-glucose was acetylated by a method first mentioned by Skraup and Koenig 1901[52]. It involves the action of acetic anhydride and sulphuric acid as catalyst on the sugar. The reaction is so rapid that little of the beta isomer is produced. The method due to Redemann and Niemann[53] involves the action of acetic anhydride and three drops of concentrated sulphuric acid as an acid catalyst on glucose. The reaction is violent and must be carefully controlled.

The pentaacetate is not isolated and acetobromoglucose is formed by passing in hydrogen bromide. A little pentaacetate was isolated from one run and on recrystallisation the melting point and specific rotation showed that it was almost all alpha-glucose-pentaacetate.

Early this year (1948) Nicholas and Smith reported[54] that a number of sugars including glucose could be acetylated in high yields using a mixture of glacial acetic acid and acetic anhydride with a little perchloric acid as catalyst. The reaction was described as rapid at room temperature and as going in high yield, but no details were given.

A private communication from Dr. W.G. Overend of Birmingham for the authors contained details of the method which can give 95% yields but usually gives yields from 60 to 90%. Using this method the yield obtained was 70.5% of α-pentaacetyl-D-glucose in the present work.

REACTION V. THE PREPARATION OF α-2:3:4:6-TETRAACETYL-D-GLUCOSYL BROMIDE (ACETOBROMOGLUCOSE).

In 1873 Colley[25] prepared acetochloroglucose by the action of acetyl chloride on the sugar. The same reaction was used by Koenigs and Knorr in 1901 to prepare acetobromoglucose[24]. Fischer improved their method in 1911 by treating β-pentaacetyl-glucose with hydrogen bromide in glacial acetic acid[55]. As the α-acetobromoglucose is much more stable than the beta isomer it has been found possible to use either α- or β-glucose-pentaacetate. Here the α-isomer was used as it is the more convenient one to prepare. Fischer and Armstrong[56] consider it inadvisable to leave the pentaacetyl-

glucose in contact with the hydrogen bromide solution for more than an hour and a half as 2:3:4-triacetyl-1:6-dibromo-glucose begins to form. The acetobromosugar is unstable and must be kept at 0°C. to prevent decomposition.

REACTION VI. THE KOENIGS AND KNORR CONDENSATION.

This reaction has been discussed under Synthesis of Glycosides, page 14. Here the condensation was carried out with excess methyl α-methyl-α-hydroxy-butyrate in dioxan solution and dry silver oxide was used to remove the halogen and anhydrous magnesium sulphate was used as the dehydrating agent. The simple Beilstein Test was used to determine when the reaction was complete.

REACTION VII. AMIDE FORMATION.

The ester group was converted to the amide by treatment with dry methanolic ammonia at 0°C. As this reagent is also a deacetylating agent the acetyl groups were removed from the 2-methyl-2-(β-tetraacetyl-glucosido)-butyramide. This reagent is not alkaline enough to hydrolyse the amide group further.

REACTION VIII. REACETYLATION OF THE 2-METHYL-2-(β-GLUCOSIDO)-BUTYRAMIDE.

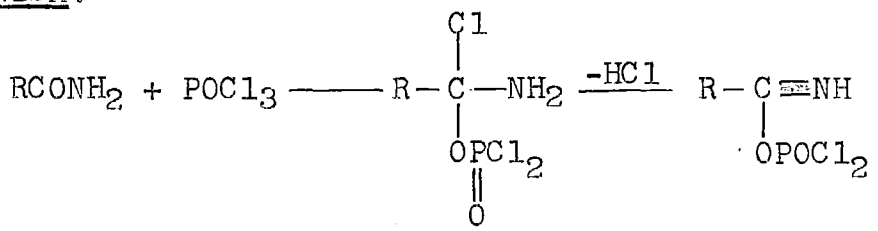
The well known method of acetylation using acetic anhydride and pyridine[57] was used, at low temperature. Any method of acetylation not interfering with the rest of the molecule could have been used as there is almost no likelihood of isomerisation of the β-glucosidic link in any of these reactions.

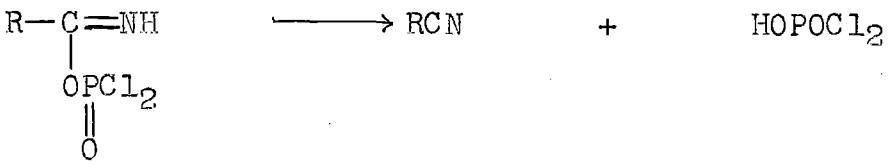
It was proposed to separate the d and l forms at this stage by fractional crystallisation as Campbell and Haworth did[43] but time did not permit this.

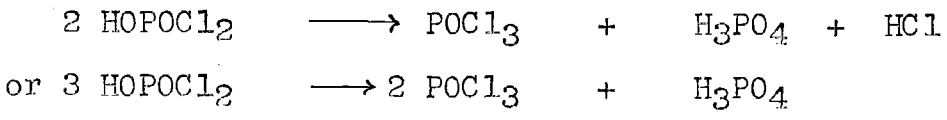
REACTION IX. THE DEHYDRATION OF THE AMIDE TO NITRILE.

It was proposed to use phosphorus oxychloride to convert the amide to the nitrile but time did not allow this step to be carried out. In a recent review[58] on the preparation of nitriles this method was reported to give good yields. For complete conversion the reaction needs only 30 - 50 mole. % of phosphorus oxychloride.

MECHANISM.







Surrey[59] claimed that the addition of sodium chloride to the above reaction mixture gives an improved yield. Fischl and Steiner[60] have used pyridine to remove hydrogen chloride formed in the reaction.

REACTION X. DEACETYLATION.

The acetyl groups can be removed with a saturated solution of ammonia in anhydrous methanol at $0^{o}C$. The otherwise convenient method of deacetylation using barium or sodium methylate could not be used here as there would be danger of these reagents attacking the nitrile group.

S U M M A R Y

1. Methyl ethyl ketone was converted to methyl ethyl ketone cyanhydrin by reaction with hydrogen cyanide in the presence of a little sodium cyanide as catalyst at 0°C.

2. Conversion of methyl ethyl ketone cyanhydrin to α-hydroxy-α-methyl butyric acid was effected by hydrolysis with fuming hydrochloric acid.

3. Esterification of this acid with methyl alcohol was carried out in the presence of anhydrous copper sulphate as an acidic catalyst and dehydrating agent, any remaining acid being esterified with diazo-methane.

4. Acetobromoglucose, prepared from glucose pentaacetate by treatment with hydrogen bromide, was condensed with the above ester in the presence of dry silver oxide and anhydrous magnesium sulphate to give methyl-2-(β-2:3:4:6-tetraacetyl-D-glucosido)-2-methyl butyrate.

5. The above compound which did not crystallise was converted to the amide by dry methanol saturated with dry ammonia at 0°C. This reagent removed the acetyl groups from the glucose residue.

6. The amide was acetylated with pyridine and acetic anhydride.

INDEX TO EXPERIMENTAL SECTION.

EXPERIMENTAL.

METHYL ETHYL KETONE CYANHYDRIN FORMATION[45].

(I) Welch and Clemo Method[61].

The following preparations were performed in a fume cupboard with efficient ventilation.

Methyl ethyl ketone (1 mole., 72 gm., 90 c.c.) was added to a solution of powdered commercial "eggs" of sodium cyanide (49 gm., 1 mole.) in 200 c.c. of water in a 1500 c.c. beaker surrounded by an ice-salt freezing mixture. Sulphuric acid (1 mole., 98 gm.) was added to water to make a 30% solution and was cooled in ice. The sulphuric acid was slowly added with stirring the temperature being kept as low as possible. The cyanhydrin separated as a white, fairly opalescent liquid as the upper layer. About an hour after adding the last of the acid the cyanhydrin was separated off with a funnel and the water layer extracted four times with 100 c.c. lots of ether. After the combined ether solution had been dried over anhydrous magnesium sulphate overnight the ether was distilled off at 35°C. The pressure was lowered to 20 m.m. and the cyanhydrin distilled over rapidly as a colourless oily liquid at 90°C.

Yield 42.4 grams. i.e. 43% of the theoretical.

$n^{19.5}$ 1.4130

c.f. n_D^{19} 1.41525 Ultee[62].

(II) Direct Combination of HCN and Methyl ethyl ketone.

Hydrogen cyanide was prepared by a modification of the method in Organic Syntheses[60].

Commercial sodium cyanide (305 gm., 6 moles.) was dissolved in as little water as possible so there would be less water for the hydrogen cyanide to dissolve in. Concentrated sulphuric acid (320 c.c., 6 moles.) was added to water (320 c.c.). These two solutions were added to a $1\frac{1}{2}$ litre flask by two dropping funnels. The hydrogen cyanide as vapor (B.P. 26°C.) was led off and passed through two calcium chloride U-tubes immersed in water kept at 35°-40°C. The hydrogen cyanide was needed dry only to ascertain how much had been produced. The vapour was passed through a vertical reflux condenser supplied with tap water (11°C.) and dipping into a weighed receiver containing the ketone surrounded by an ice-salt freezing mixture.

Yield of hydrogen cyanide 119.9 grams (74.0% of the theoretical).

To methyl ethyl ketone (315 c.c., 3.5 moles., 252 gm.) in a bolthead flask was added a solution of 3 gm. of sodium cyanide in 10 c.c. of water. The flask was weighed then surrounded by an ice-salt freezing mixture. Hydrogen cyanide was added slowly but the addition was periodically stopped to allow the reaction mixture to cool. The reaction vessel remained in ice overnight and next day the sodium cyanide was filtered off. To the deep red solution concentrated hydrochloric acid was added drop by drop with stirring until the solution was just acid to congo red paper. It was then distilled. The main fraction came over at a bath temperature of 94°C., boiling at 87°C. 15 m.m. pressure.

Yield 273.5 gm. i.e. 79.0% of theoretical calculated on methyl ethyl ketone.

n^{20} 1.4140

c.f. n_D^{19} 1.41525 Ultee[62].

PREPARATION OF α-HYDROXY-α-METHYL BUTYRIC ACID[46].

Methyl ethyl ketone cyanhydrin (99 gm., 1 mole.) was refluxed gently with 450 c.c. of concentrated hydrochloric acid for 6 hours on a water bath. From time to time the mixture was swirled gently. A copious white precipitate of ammonium chloride formed. The mixture was evaporated to a third of its original volume on the water bath and the ammonium chloride which separated on cooling was filtered off. The filtrate was extracted with ether in a separating funnel and the ethereal extracts dried by standing over anhydrous magnesium sulphate overnight. The ether was distilled off at 35°, then the last traces were removed at low pressure. The contents of the flask were poured into a weighed basin where almost colourless needles of the acid soon appeared. The acid was very hygroscopic.

Yield 110.3 grams. i.e. 93.5% of the theoretical.

M.P. 70°C.

On two recrystallisations from petrol ether (B.P.40°-50°) colourless needles melting at 72.5°C. were obtained.

c.f. M.P. 72°-73°C. Heilbron and Bunbury[64].

About 5 grams of the acid in 30 c.c. of distilled water were left in contact with excess silver carbonate for

three weeks. On filtering and evaporating in a vacuum desiccator rosettes of almost colourless crystals of the silver salt formed. These were rinsed with distilled water and dried in a desiccator. A sample of the silver salt was weighed in a platinum boat.

This was heated over a Fischer burner gently at first and later at red heat in a furnace made of a horizontal piece of silica tubing 20 cm. long and 2.5 cm. in diameter.

0.01724 gm. of silver salt gave 0.00827 gm. of silver.

% silver = 47.97% (theoretical 48.10%).

ESTERIFICATION OF α-HYDROXY-α-METHYL-BUTYRIC ACID.

(I) ALCOHOLYSIS.

In one trial run[65] 10 c.c. (9.2 gm.) of methyl ethyl ketone cyanhydrin and 50 c.c. of pure ethyl alcohol containing 5 gm. of dry hydrogen chloride were refluxed on a glycerol bath at 100° -115° for 6 hours. The white precipitate of ammonium chloride was filtered off and the solution stood over excess silver carbonate with occasional stirring for about an hour. This was then filtered off and excess alcohol removed at low pressure at less at 45°C. On raising the temperature only a trace of distillate came over and a white amorphous solid appeared in the flask. This suggested, incorrectly as it turned out, that the ester was easily decomposed so it was decided to use diazo-methane as an esterifying agent. The white amorphous solid charred without melting at 197° so it was not angelic or tiglic acids. It was most likely the lactide but no further time was spent on this.

(II) WITH DIAZO-METHANE[48].

The advantages of diazo-methane are that almost the only byproduct is nitrogen and the reaction can be carried out in dry ether which is easily removed.

Methyl urea (10 gms., 0.135 mole.) was converted to the hydrochloride with 20 c.c. concentrated hydrochloric acid (0.2 moles.) in a 600 c.c. beaker as the reaction mixture froths. Sodium nitrite (14 gm., 0.2 moles.) was added a little at a time with stirring at -10°C. After a short time the mixture was poured into ice (120 gm.) and concentrated sulphuric acid (11.2 c.c., 0.2 moles.) when the nitrosomethylurea rose to the surface as a foamy micro-crystalline solid. This was filtered at the pump, washed with ice water and pressed on the filter paper and was dried in a vacuum desiccator in a cool place. Nitrosomethylurea is not very stable and may explode is kept for some weeks.

Yield 10.61 gms. i.e. 76.2% of the theoretical.

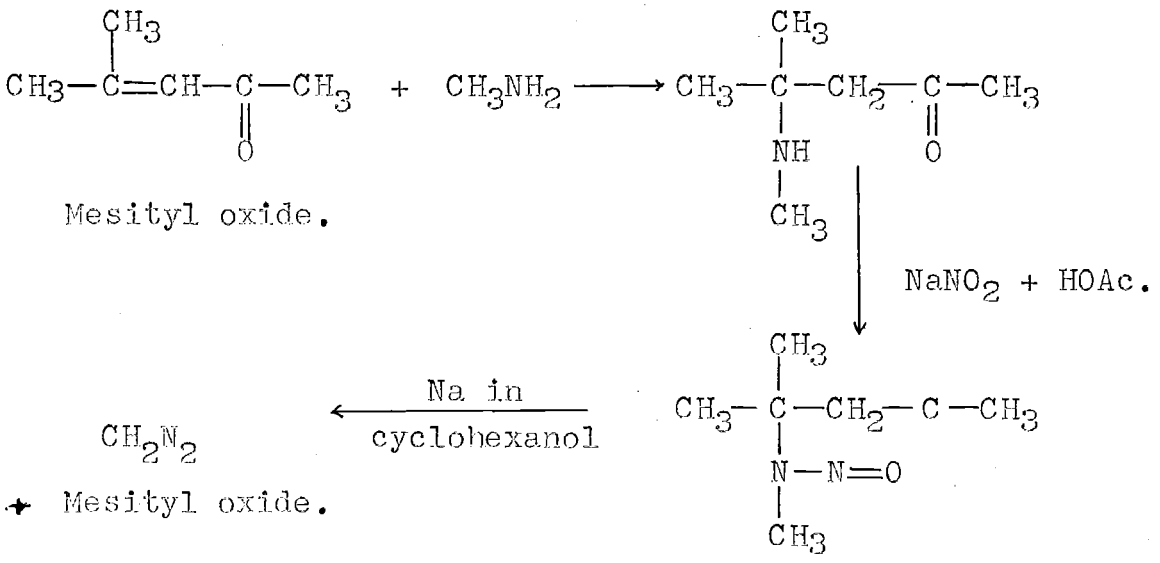
Another preparation gave an 83.6% yield.

Conversion to Diazo-methane.

This was carried out in a fume cupboard with an efficient fan. In a tall 800 c.c. beaker 180 c.c. of 40% potassium hydroxide solution were cooled to 0°C. 500 c.c. of ether were added and when the temperature reached -10°C. with an ice-salt mixture 60 gm. of nitrosomethylurea were added in small portions with stirring. After an hour the bright yellow ethereal solution of diazomethane was poured off and other 200 c.c. of ether added. The combined ethereal solutions were dried for three hours over potassium

hydroxide pellets and decanted off. Even in ether solution diazomethane decomposes slowly so the solution was used immediately.

Adamson and Kenner's method of preparing diazohydrocarbons[66] was applied to diazomethane using mesityl oxide prepared in 65.25% yield from acetone according to the method in Organic Syntheses[67].



Although the conditions described were adhered to, the yield of nitrosoaminoketone was only 34% which compared unfavourably with the 70-80% yields claimed by the authors and with the yields of nitrosomethylurea. This method was not given any further consideration.

Esterification with Diazo-methane.

60 gms. (0.583 moles.) of Nitrosomethylurea were treated with alkali and the resulting ethereal solution of diazo-methane dried over pellets of potassium hydroxide.

To this solution was added 30 gms. (0.254 moles.) of α-hydroxy-α-methyl-butyric acid previously dried in a vacuum desiccator for two days over phosphorus pentoxide. The solution remained bright yellow and effervesced as before when a few additional crystals of the acid were added. This confirmed that there was an excess of diazo-methane present. The flask was stoppered with a cork with a bend and calcium chloride tube and stood over night. Next day the ether was removed at atmospheric pressure and 35°C. The pressure was lowered to 20 m.m. and the main fraction distilled at 45°. The boiling point of the methyl ester[68] is 152°C. at 760 m.m.

$$L_m = 21 \times (152 + 273) \quad \text{(by Trouton's Rule.)}$$

and $$\text{Log } \frac{760}{20} = \frac{21 \times 425}{1.987 \times 2.303} \cdot \frac{(425 - T)}{(425 \times T)}$$ (Clausius-Clapeyron Equation).

$\therefore$ B.P. = 43°C. at 20 m.m. pressure.

which agreed fairly well with the actual boiling point considering the approximate nature of Trouton's Rule.

Yield 24.6 gm. i.e. 73.5% of the theoretical.

Found %-OCH_3 23.13% by the Zeisel method.

Theoretical %-OCH_3 23.48%

The discrepancy was probably due to insufficient drying of the solution of diazo-methane.

(III) WITH ANHYDROUS COPPER SULPHATE[51].

Recrystallised acid (45 gm., ·381 moles.), anhydrous methanol (180 c.c., 4.5 moles.) and 20 gm. of anhydrous copper sulphate were refluxed for 8 hours on a water bath, moisture

being excluded with a calcium chloride tube. The reaction mixture was then stirred with 300 c.c. of distilled water and the ester extracted with a total of 600 c.c. of ether. The ethereal extract was dried over night over anhydrous magnesium sulphate and the ether removed at 35°. The pressure was then lowered to 20 m.m. and held there to remove the last traces of ether. On raising the temperature the ester distilled from 40° to 46°.

Yield 49.7 gm. or 98.8% of the theoretical.

Another preparation gave a yield of 76.0% of the theoretical.

Found %-OCH_3 21.92%

Theoretical %-OCH_3 23.48% for $C_4H_9O \cdot COOCH_3$.

After distillation of 30 gm. of ester over 5 gm. of phosphorus pentoxide[69] the per cent. methoxy was determined.

Found %-OCH_3 23.32%

Theoretical %-OCH_3 23.48% for $C_4H_9O \cdot COOCH_3$.

PREPARATION OF α-1:2:3:4:6-PENTAACETYL-D-GLUCOSE[54].

Commercial pure α-D-glucose was used.

M.P. 146°C. (uncorrected)

$[\alpha]_D^{16^\circ}$ = 112.0° after 5 minutes (C = 4.060 in water).

Anhydrous glucose (50 gm., 0.277 moles.) was warmed to 30°C. in a mixture of 150 c.c. glacial acetic acid (2.62 moles.) and 150 c.c. acetic anhydride (1.59 moles.) in a 1500 c.c. beaker. This was then stood in a bath of tap water and 1.5 c.c. of AnalaR 60% perchloric acid added. On stirring, the temperature rose and reached 75°C. at one time, but was mainly below 40° and all the glucose dissolved. After standing for a quarter of an hour the beaker was filled with tap water and the glucose pentaacetate came down. This was allowed to stand for two hours and was filtered and washed at the pump. It was then mixed with distilled water and stood for two days when it was filtered and dried in a vacuum oven at 50°C. A sample was recrystallised from absolute alcohol, This method produced mainly the alpha isomer.

Yield 76.3 gm. i.e. 70.5% of the theoretical.

M.P. 110°C (uncorrected)

c.f. M.P. 112°-113°C Hudson and Dale[70].

$[\alpha]_D^{20^\circ}$ = 95.01° (C 1.5156 chloroform)

c.f. $[\alpha]_D^{20^\circ}$ = 101.6° ($CHCl_3$) Hudson and Dale[70].

The details of this method due to Nicholas and Smith were not available when the above preparation was carried out but they proved to be identical except that 200 c.c. of glacial acetic acid and 200 c.c. of anhydride were used. Adherence to their conditions might have improved the yield.

∝-2:3:4:6-TETRAACETYL-D-GLUCOSYL BROMIDE[55] (ACETOBROMOGLUCOSE).

Finely powdered ∝-D-glucose pentaacetate (100 gm., 0.256 moles.) was added to 100 c.c. of glacial acetic acid containing dry hydrogen bromide (27 gm., 0.3 moles.) at 0°C. Solution was speedily effected by shaking. The stoppered flask was stored in the refrigerator over night. Chloroform (200 c.c. B.P.) was added and the solution poured in a thin stream into ice water. The flask was rinsed out with a further small quantity of chloroform and the combined solution was stirred. The chloroform layer was then separated off and stood over a few granules of calcium chloride until clear. The solution was then decanted from the drying agent into a large basin and 50 c.c. of petrol ether (B.P. 40° - 60°) added. This caused the syrup to crystallise when stored in the refrigerator for two days.

Yield 62 gm. i.e. 58.9% of the theoretical.

M.P. 85° C. (uncorrected)

On recrystallisation from ether and petrol ether (B.P. 40° - 60°) a sample crystallised in rosettes of needles.

M.P. 88.5°C. (uncorrected)

c.f. 88° - 89°C. Koenigs and Knorr[24].

ALTERNATIVE PREPARATION OF ACETOBROMOGLUCOSE[53].

To a mixture of 44 gm. of anhydrous D-glucose (0.244 moles.) and 186 c.c. (200 gm., 1.972 moles.) of acetic anhydride in a 700 c.c. flask were added three drops of concentrated sulphuric acid from a pipette. On swirling the contents a considerable amount of heat was evolved and the flask was cooled under the tap. The sulphuric acid acts as an acid catalyst and if too much is added the reaction becomes unmanageable.

The flask was then fitted with a condenser and heated on a boiling water bath for two hours. Then about 80 c.c. of glacial acetic acid - acetic anhydride mixture were distilled off at 20 m.m. and 50°C. bath temperature. Most of the acetic acid would be expected to come off as its boiling point is 118°C. compared with that of acetic anhydride which is 138°C. The flask was cooled in ice and 81 gm. (1 mole.) of dry hydrogen bromide passed in with shaking. The sealed flask was kept in the refrigerator overnight. The mixture was then poured into 2 litres of ice water and 350 c.c. of chloroform added. This solution was stirred and separated and the aqueous layer extracted with two 100 c.c. lots of chloroform. The combined chloroform solution was shaken with a few granules of calcium chloride until clear, then the solution evaporated down at ordinary temperatures and 20 m.m. pressure. The syrup was poured into a crystallising dish when it crystallised on the addition of petrol ether (B.P. 40° - 60°).

The crude product was dissolved in the minimum amount of pure methanol and the solution was poured into ice water.

It crystallised in fine white crystals. These were filtered at the pump and spread on a tile in a vacuum desiccator over P_2O_5, in a refrigerator.

Yield 60 gm. i.e. 59.7% of the theoretical.

M.P. 85° (uncorrected) c.f. page 43.

THE CONDENSATION OF ACETOBROMOGLUCOSE WITH METHYL-α-HYDROXY-α-METHYL-BUTYRATE.

Pure Methyl α-hydroxy-α-methyl-butyrate (70 gm., 0.530 moles.), silver oxide (50 gm., 0.215 moles.) dried in a vacuum desiccator over phosphorus pentoxide for ten days and anhydrous magnesium sulphate (30 gm.) were mixed in a flat-bottomed 1500 c.c. flask. Dry dioxan (250 c.c.) was added and the flask stoppered with a two holed rubber stopper fitted with a motor driven stirrer with a mercury seal and with a bend attached to a calcium chloride tube. After stirring for half an hour acetobromoglucose (60 gm., 0.146 moles.) was added little by little. When the condensation had proceeded for twenty four hours a few drops of the reaction mixture were removed and filtered. A clean copper wire dipped in the filtrate was heated in the Bunsen flame. The absence of a green flame showed no halogen was present, (i.e. a negative Beilstein test) indicating that no acetobromoglucose remained.

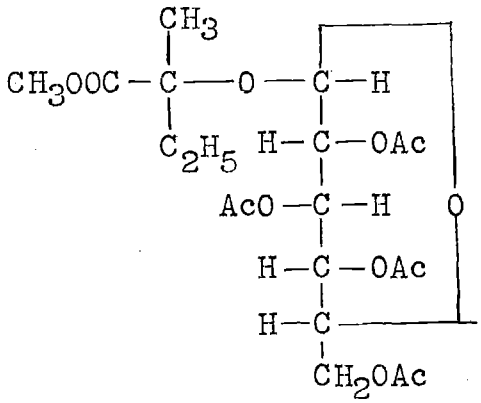
The solids were then removed by filtration at the pump and washed with 300 c.c. of hot dioxan. The combined dioxan solution was a pale brown colour. On stirring for a few minutes with 0.5 gm. of activated charcoal and filtering the solution became a pale yellow.

Dioxan was removed at 30° to 35° C. under reduced pressure (20 m.m.) using a filter pump, then the flask was connected through a receiver and trap with a rotary vacuum pump and the last of the ester removed at 0.5 m.m. pressure and 30° C.

This left 35 gm. of a thick pale yellow syrup which reduced Fehling's solution, indicating the presence of 2:3:4:6-tetraacetyl-D-glucose. Anhydrous calcium sulphate would have been more efficient in preventing the formation of this than was anhydrous magnesium sulphate. No Beilstein Test for halogen could be obtained.

Found %-OCH_3 2.43%

Theoretical %-OCH_3 6.71% For the compound below.



Methyl α-methyl-α-(β-2:3:4:6-tetraacetyl-D-glucosido) butyrate.

The acid equivalent of the condensation product was determined by a type of acetyl determination. (See appendix of analytical methods).

Number of ester groups found 4.84, 4.98.

Theoretical 5,

made up of four acetyl groups and the ester group on carbon one.

The syrup could not be made to crystallise although all the common solvents were tried, and solutions and the syrup itself were cooled in a freezing mixture of solid

carbon dioxide and alcohol. Ethyl alcohol and ethyl acetate were not used as probably there would be the danger of alkyl exchange with ethyl replacing methyl in the above compound.

The presence of d and l forms and the unsymmetrical shape of the molecules may have been responsible for its reluctance to crystallise.

AMIDE FORMATION.

20 gm. of the syrupy condensation product were dissolved in 250 c.c. of anhydrous pure methanol saturated with dry ammonia at 0°C. and the flask sealed and shaken and then stored in the refrigerator for five days when the solution was again saturated with dry ammonia and replaced for a further five days. The methanol and ammonia were then removed at 30° bath temperature under reduced pressure. The syrup was extracted with a total of 500 c.c. of warm ethyl acetate which removed the acetamide and the traces of ethyl acetate were removed at 40° bath temperature and 20 m.m. pressure. This left 12.5 gm. of a brown syrup which would contain d and l unacetylated amides and some free glucose from the deactylation of 2:3:4:6-tetraacetyl-D-glucose. The amide did not crystallise. This was not unexpected as Fischer and Anger[71] had difficulty in crystallising the corresponding compound in the Linamarin Synthesis.

RE-ACETYLATION OF THE AMIDE.

The amide syrup (about 10 gm.) was shaken with 35 c.c. of pyridine and 23 c.c. of acetic anhydride and stood at room temperature for 10 days. The mixture was then dissolved in chloroform and shaken with cold distilled water and then dilute sodium bicarbonate solution. After washing again with distilled water it was further washed with a total of a litre of 5% copper sulphate solution to remove the pyridine. The chloroform solution was then dried over anhydrous magnesium sulphate. A small portion was evaporated down under reduced pressure leaving a glassy mass. The main fraction was allowed to evaporate to dryness in a desiccator and then triturated with a mixture of pure ethyl alcohol and ether. This solution which smelled very faintly of pyridine was allowed to evaporate slowly and gave rosettes of colourless needles.

M.P. 99° - $100^{\circ}C$ (uncorrected).

ANALYTICAL METHODS.

SEMI-MICRO METHOXYL DETERMINATION[72].

A modified Zeisel determination based on that described by Hickinbottom was used. Alkoxy groups may be removed from esters or ethers as alkyl iodides on boiling with strong hydriodic acid (S.G. 1.7, B.P. 135°C.) A small trap containing a suspension of washed red phosphorus in distilled water removes any hydrogen iodide. Dry carbon dioxide carries off the alkyl iodide which reacts in two traps with 2% alcoholic silver nitrate giving a precipitate of silver iodide which is weighed directly in a sintered glass crucible.

$$\% - OCH_3 = \frac{\text{Weight of AgI} \times 0.1322}{\text{Weight of sample}} \times 100$$

Acetyl groups present do not affect this determination[73].

THE DETERMINATION OF THE NUMBER OF CARBOXYL GROUPS[74].

This is a type of acetyl determination.

About 20 m.g. of the compound were weighed accurately into a glass capsule and placed in a 25 c.c. conical flask. 10 c.c. of standard $\frac{N}{10}$ alkali was added and the flask warmed at 30°C. until the contents were homogeneous. After three hours with intermittent shaking the excess alkali was titrated with standard $\frac{N}{10}$ sulphuric acid using phenolphthalein as indicator. Knowing the molecular weight of the compound the number of carboxyl groups could be determined.

PURIFICATION OF REAGENTS.

The commercial methyl ethyl ketone (Hopkins and Williams) was used with no further purification.

B.P.	79.0°C.	at 760 m.m.	
B.P.	79.6°C.	at 760 m.m.	I. C. T.
n^{19}	1.3801		
n_D^{20}	1.3791	I. C. T.	

METHANOL.

Commercial absolute methanol was stood over calcium oxide for ten days then refluxed and distilled off. Iodine (5 gm.) was dissolved in a little of the main fraction and magnesium turnings (15 gm.) were added. When the reaction had subsided the reminder of the main fraction was added and refluxed for eight hours.

The alcohol was then slowly distilled through a long fractionating column packed with glass beads. The whole apparatus was in glass. A small first fraction was discarded.

B.P.	64.8°C.		
B.P.	64.5°C.	I. C. T.	
	$n^{20°}$	1.3290	
c.f.	$n_D^{20°}$	1.3290	I. C. T.

ETHER.

Ether (S.G. 0.720) was dried over calcium chloride, filtered and stored over sodium wire away from the light.

ACETONE.

Acetone (1 litre B.P.) was shaken with 20 ml. N. sodium hydroxide solution and 7 grams of silver nitrate for a day then decanted off and stood over excess fused calcium chloride. It was then slowly distilled through an efficient fractionating column.

n^{19°	1.3615		B.P.	56.2°C.	
$n_D^{20^\circ}$	1.3591	I. C. T.	B.P.	56.1°C.	I. C. T.

CHLOROFORM.

Chloroform (B.P.) was washed six times with water by shaking vigorously. It was then dried over calcium chloride and distilled off phosphorus pentoxide twice.

B.P.	61.0°C.		n^{20}	1.4460	
B.P.	61.2°C.	I. C. T.	n_D^{20}	1.4467	I. C. T.

PYRIDINE.

Commercial pyridine was refluxed over excess anhydrous barium oxide and fractionated through a long column. The main fraction boiled at 115°C.

n^{20}	1.5072	
n_D^{20}	1.5090	I. C. T.

ETHANOL.

Commercial absolute alcohol was treated as for methanol.

n^{20}	1.3616		B.P.	78.3°C.	
n_D^{20}	1.3610	I. C. T.	B.P.	78·5°C.	I. C. T.

DIOXAN.

Technical dioxan was stored over calcium chloride filtered and distilled under reduced pressure. Care was taken that the flask did not become dry as an explosion of the peroxides might then have resulted.

n^{20°	1.4220
$n_D^{20^\circ}$	1.42228 [75]

SILVER OXIDE.

AnalaR barium hydroxide (100 gm.) was dissolved in distilled water (1000 c.c.) and the barium carbonate filtered off. Silver nitrate (100 gm.) was dissolved in 500 c.c. of distilled water. The hot solutions were mixed with stirring and the precipitate of silver oxide was washed with hot distilled water until only a faint test for barium could be obtained in the washings on adding a few drops of strong sulphuric acid to a sample.

The silver oxide was then rinsed with pure acetone and dried in a vacuum oven at 80°C. for three days.

The resulting oxide was powdered in a mortar, sieved through a fine mesh bolting silk and stored in a tightly stoppered brown bottle.

GLACIAL ACETIC ACID SATURATED WITH HYDROGEN BROMIDE[76].

Red phosphorus (12 gm.) was mixed to a sludge with hydrobromic acid (30 c.c.) in a 250 c.c. distilling flask fitted with a dropping funnel the stem of which extended to just above the sludge. Bromine (40 c.c., 120 gm.) was dropped onto the sludge and the gas evolved was passed through a U-tube containing glass beads and moist red phosphorus and then a U-tube containing calcium chloride. To prevent sucking back the gas was passed through a large splash-head the stem of which dipped into the glacial acetic acid. The glacial acetic acid was not surrounded by the ice bath until some of the hydrogen bromide had dissolved to prevent the acetic acid freezing.

METHANOLIC AMMONIA.

Dry ammonia was produced by boiling .880 ammonia in a 500 c.c. flask fitted with a vertical reflux condenser connected with a tall soda-lime tower. The ammonia was bubbled through pure methanol in a flask surrounded by ice until no more dissolved.

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